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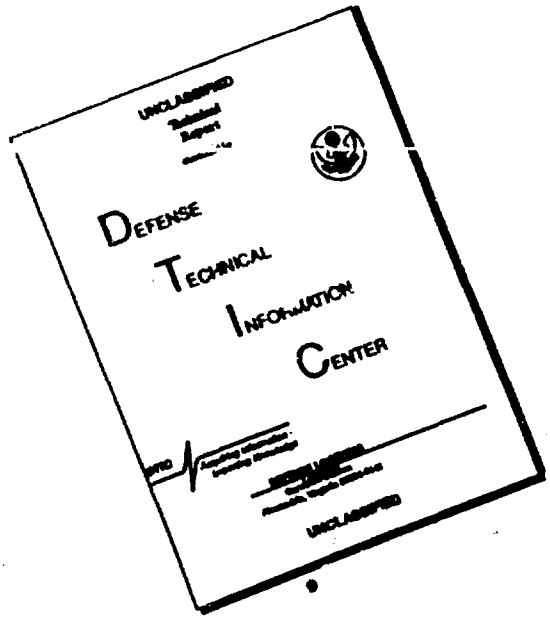

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EFFECT OF SOME CHEMICALS ON MICROFLORA OF GRAPE JUICE

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F. I. Skoropad, V. V. Kotelev and Kh. V. Al'man

Sorbic acid is used in foreign countries as a preservative for canning perishable foodstuffs(4,5). Research work was also carried out in the Soviet Union in order to adopt sorbic acid as a preservative(1,2,3). However, sorbic acid is not used widely in the approved quantities because of its high cost. Moreover, sorbic acid is not a sufficiently reliable preservative of grape juice. Therefore, the study of the effect of chemical preparations and antibiotics on the microflora of grape juice is an urgent problem.

The object of this work was to find the most effective preparation that would satisfy the requirements of a preservative. For this purpose we studied the following substances: pyromucic acid, methyl and ethyl ethers of 5-nitropyromucic acid (menips and enips), 5-chlorpyromucic acid, 5-methylpyromucic acid and others. These substances were synthesized from furfurole in the Institute of Chemistry of the Academy of Sciences, Moldavian SSR, (Academician A. V. Lazur'yevskiy's laboratory).

Moreover, we studied the antibiotics nystatin, grisin, polymyxin, biocillin and others.

The chemical substances and antibiotics were tested under laboratory and semi-industrial conditions.

I. Laboratory Experiments

In order to make a preliminary study of the effectiveness of the preparations in comparison with sorbic acid, we carried out short experiments according to the following method.

Sterile grape juice was poured into test tubes in the amount of 4-4.5 ml. The preparations were introduced into the juice by the method of serial dilutions. In order to obtain the necessary concentration the juice in the test tubes was inoculated with one drop of 1-billion microorganism suspension of the following cultures: Saccharomyces cerevisiae, Saccharomyces vini, Aspergillus niger, Mucor sp. and a mold fungus isolated from grape juice. The inoculated test tubes with the juice were kept in a thermostatically controlled chamber at a temperature of 28-30°C for five days. The test tubes with the juice were examined daily. Juice without the preparation served as a control. The juice was considered spoiled if the following conditions appeared: gas formation, sediment, cloudiness, appearance of a film within or on the surface of the juice. After five days, the juices from the test tubes which did not show spoilage were inoculated into a sterile grape juice in order to ascertain more precisely the inhibiting effect of the preparation.

Table 1 shows the results of our verification of the effect of the preparation on the microflora of grape juice*.

It can be seen from Table 1 that pyromucic acid, 5-chlorpyromucic acid, 6-methylpyromucic, 3-chlorazol and 2-chloranilide do not inhibit the development of the microflora which causes grape juice to spoil. As can be seen, the juice with the above-mentioned substances which was inoculated with microorganisms fermented during the 24 and 48 hour periods.

The juice with the menips and enips preparations in the amount of 0.012-0.10% remained transparent for five days, and with sorbic acid in a concentration of 0.012% it fermented on the second day.

In a similar way we studied the threshold concentration of the menips and enips preparations and sorbic acid inhibiting the development of test cultures of the microorganisms. The results of these studies are shown in Table 2.

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*All Tables show average data of 2-3 repeated tests.

It can be seen from the Table that these preparations menips at a concentration of 0.003%, enips at 0.006%, and sorbic acid at 0.05% inhibit the development of yeast cultures. A higher concentration is necessary for the mold fungus.

In addition to testing the preparations with artificial inoculation we also conducted experiments on the effect of the preparations on a natural insemination of juice with microorganisms. For this purpose we used grape juice which had been in cold storage in tanks for five months. The total natural insemination of the juice was 100,000 cells in 1 ml of the juice.

A definite amount of the preparations was introduced into each 250 ml-capacity conic flask containing 100 ml of grape juice. The flasks with the juice were kept at a temperature of 28-30°C for 24 hours and at room temperature for the remaining time. The results of the experiment are shown in Table 3.

It can be seen from the Table that the most active preparations are enips and menips. The juice with the menips preparation in the amount of 200 mg/l preserved for five months and longer.

Sorbic acid in the amount of 200 mg/l inhibited the fermentation of the juice for 9 days, but 100 mg/l proved ineffective.

In order to determine the combined effect of preparation enips and various temperatures of pasteurization on the preservation of juice, we conducted an experiment in conic flasks of 250 ml. The results of this experiment are shown in Table 4.

It can be seen from the Table that it is more effective to use a combination of preparation enips in the amount of 50 mg per one liter of juice and heating for five minutes at a temperature of 65-70°C, which preserved the juice in the course of one month.

Besides chemical preparations, we studied the effect of antibiotics on the microflora of grape juice. The experiments were carried out on sterile grape juice following the method adopted for chemical preparations. The results of the experiments are given in Table 5.

Table 1

Effect of Chemical Preparations on the Microflora of Grape Juice

Period of storage, days	Control	Tested substances																													
		5-methyl-pyromucic acid				Pyro-mucic acid				5-chlor-pyro-mucic acid				Methyl ether of pyromucic acid				Salicy-lani-lide				5-nitro-methyl-pyromucic acid				Unips				Menips				Sorbic acid			
		0,1	0,05	0,025	0,012	0,1	0,05	0,025	0,012	0,1	0,05	0,025	0,012	0,1	0,05	0,025	0,012	0,1	0,05	0,025	0,012	0,1	0,05	0,025	0,012	0,1	0,05	0,025	0,012	0,1	0,05	0,025	0,012	0,1	0,05	0,025	0,012
1st	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2nd	+	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	+
3rd		−	+			−												+								−	−	−	−	−	−	−	−	−	−	−	
4th		−				−												−								−	−	−	−	−	−	−	−	−	−	−	
5th		+				+																				−	−	−	−	−	−	−	−	−	−	−	

Note: − sterile; + fermented

It can be seen from the Table that nystatin in the amount of 100 mg/l of juice is the most active and inhibits fermentation for 20 days. Other antibiotics, such as biocillin, polymyxin, mycerin No. 2886, 2339, 2789, did not yield positive results under the same conditions. However, nystatin noticeably changes the odor of the juice, which is in agreement with the experimental data of the laboratory of antibiotics of the TsNIIKOP (Central Research Institute, Canning and Vegetable Drying Industry).

Table 2

Threshold Concentration of Preparations Inhibiting the Development of the Test Cultures of Microorganisms

Culture of microorganisms	Preparations, %													
	Enips					Menips						Sorbic Acid		
	0,1	0,05	0,025	0,012	0,006	0,1	0,05	0,025	0,012	0,008	0,003	0,1	0,05	0,025	0,012
Saccharomyces cerevisiae	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
S. vini	–	–	–	–	–	–	–	–	–	–	–	–	–		
S. vini 85	–	–				–	–	–	–			–	–	–	
S. vini 92	–	–	–	–	–	–	–	–	–			–	–	–	
Mold fungus from grape juice	–	–	–	–		–	–	–	–			–	–	+	–
Mucor sp.	–	–	–			–	–	–	–			–	–		
Aspergillus niger	–	–	–	–		–	–	–	–			–	+	+	

Notes: – bactericidal characteristic; + bacteriostatic characteristic.

Table 3

Preservation Effect of Some Preparations on Grape Juice

Preparations	Concentration of prep. mg/l	Preservation of juice, days											
		3	4	5	6	10	15	20	25	30	40	48	5 mos.
Control without preparation . . .	—	+											
Corinal	30	—	—	—	+								
Nitrofungin . . .	30	—	—	+									
	60	—	—	—	—	—	+						
Sorbic Acid . . .	100	—	+										
	200	—	—	—	—	+							
Enips	50	+											
	100	—	—	—	—	—	+						
Menips	100	—	—	—	—	—	—	—	—	—	—	+	
	200	—	—	—	—	—	—	—	—	—	—	—	—

Note: + fermented; - sterile

Table 4

Combined Effect of Preparation Enips in a Concentration of 0.005% and Heating in the Course of Five Minutes at Various Temperatures on the Preservation of Grape Juice

Variant	Pasteurization temp., degrees.	Storage period of juice, days									
		2	3	4	6	8	10	12	20	28	30
Control(without prep.)	50	+									
Control(without heat.)	—	—	+								
Juice + enips	50	—	—	+							
Control(without prep.)	60	—	+								
Juice + enips	60	—	—	—	—	—	—	+			
Control(without prep.)	65	—	—	—							
Juice + enips	65	—	—	—	—	—	—	—	—	—	+
Control(without prep.)	70	—	—	+							
Juice + enips	70	—	—	—	—	—	—	—	—	—	+

Note: - Fermented; + Sterile

Table 5

Storage Period of Grape Juice Depending on the Antibiotic Used (in a concentration of 100 mg/l).

Variant	Duration of storage, days.						
	1	2	3	4	5	10	20
Grape juice (control).	+						
Grape juice + antibiotic 2886.	−	+					
Grape juice + antibiotic 2339.	−	+					
Grape juice + antibiotic 2789.	−	+					
Grape juice + biocillin	−	+					
Grape juice + polymyxin	−	+					
Grape juice + grisin	−	−	+				
Grape juice + mycerin	−	+					
Grape juice + nystatin.	−	−	−	−	−	−	−

Note: − Fermented; + Sterile

II. Studies on Effective Preparations Under Semi-Industrial Conditions

Preparations which were found to be most effective under laboratory conditions were further tested under semi-industrial conditions. For this purpose, the laboratories of the Kishinev Fruit Canning Plant conducted two variants of the experiment. They used grape juice which was kept in cold storage (tank) for five months with a total natural insemination of 27 million cells in 1 ml of juice.

In the first variant, the juice was pasteurized for five minutes at a temperature of 60° and in the second variant -- five minutes at 75°. After the necessary quantities of the preparations have been added, the juice was poured into glass jars 83-1, covered with air-tight lids and sealed on a sealing machine.

The results of the experiment are given in Table 6.

Table 6

Effect of Preparations Menips and Enips and Short-Duration Pasteurization on the Preservation of Grape Juice

Variant	Concentration of prep., %	5 min. pasteurization at a temperature	Preservation of juice, days						
			1	2	3	10	20	24	8 mos.
Control (without prep.).	—	75°C	—	—	+				
Juice + menips	0,01	.	—	—	—	—	—	—	—
	0,01	.	—	—	—	—	—	—	—
	0,02	.	—	—	—	—	—	—	—
	0,02	.	—	—	—	—	—	—	—
Juice + enips	0,01	.	—	—	—	—	—	—	—
	0,01	.	—	—	—	—	—	—	—
	0,02	.	—	—	—	—	—	—	—
	0,02	.	—	—	—	—	—	—	—
Control (without prep.).	—	80°C	—	+					
Juice + menips	0,01	.	—	—	—	—	—	—	—
	0,01	.	—	—	—	—	—	—	—
	0,02	.	—	—	—	—	—	—	—
	0,02	.	—	—	—	—	—	—	—
Juice + enips	0,01	.	—	—	—	—	—	—	—
	0,01	.	—	—	—	—	—	—	—
	0,02	.	—	—	—	—	—	—	—
	0,02	.	—	—	—	—	—	—	—

The Table shows that the control specimens without the preparations fermented on the second-third day while juices with preparations enips and menips in the amount of 100-200 mg per one liter remained unchanged in the course of eight months.

In the laboratory of the Kalarash Fruit Canning Plant, a semi-industrial experiment was conducted with preparations menips and β-propiolactone. For this purpose they use plum compote, apple and grape juice -- semi-finished products. For this study, the compote was prepared according to technological instructions and the juices were obtained after squeezing and coarse filtration. After heating the juices to a fixed temperature, a weighed or measured amount of the preparation

was introduced. The juice was poured into glass jars 83-1, covered with air-tight lids and sealed with a manual sealer. The experimental products were stored in the laboratory at a temperature of 24-28°C. All experiments were carried out twice and repeated 2-6 times.

Table 7

Storage Time of Juices and Compote Depending on the Pasteurization Temperature and the Amount of Introduced Preparations

Variant		Pasteurization temperature, 15 minutes	Storage period, days		
			Juices		Plum compote
			Grape	Apple	
Control (without prep.)		50°C	2	1	2
		60°C	8	6	7
		70°C	19	60 &>	
Menips	0,005%	—	2		
	0,01%	—	2	2	2
	0,02%	—	2	2	2
Menips	0,005%	50°C	15		
	"	60°C	60 &>		
	"	70°C	60 &>		
	0,01%	50°C	25	16	
	"	60°C	60 &>	90 &>	25
	"	70°C	60 &>	90 &>	
	0,02%	50°C	30		
	"	60°C	60 &>	90 &>	270 &>
		70°C	60 &>		
β-propiolactone II					
	0,1/100*	—	1	1	
	0,2/100	—	1	1	
	0,1/100	50°C	45 &>	240 &>	5
	0,1/100	60°C	45 &>	"	270 &>
	0,2/100	50°C	45 &>	"	6
	0,2/100	60°C	45 &>	"	270 &>
	0,2/100	70°C			270 &>
	0,05/100	50°C			6
	0,05/100	60°C			270 &>

*Amount of β-propiolactone II milliliters per 100 ml of juice.

The data on the dependence of the length of storage on the preparations used and the temperature of pasteurization are shown in Table 7, from which we can see that pasteurization at 50° does not preserve grape juice. A combined use of preparation menips in the amount of 50 to 250 mg/l and pasteurization in the course of five minutes at a temperature of 60-70° preserves the juice for 60 or more days. β-propiolactone in the amount of 0.1 ml per 100 ml of juice and pastuerization in the course of five minutes at 50°C preserves grape juice for 45 or more days.

Menips did not yield positive results in apple juice in a concentration of 0.01% + pasteurization in the course of five minutes at 50°C. β-propiolactone preserves apple juice for eight or more months.

Our analysis of the data on the plum compote has shown that pasteurization temperature of 50° in combination with β-propiolactone in various concentrations is ineffective. The combination of pasteurization at a temperature of 60-70° and various volumes of β-propiolactone preserves the product within 9 and more months.

The MIEMG* (Kishinev) Institute for 10 months has been conducting experiments on the toxicologic properties of preparation menips on animal organism (rats, rabbits). Their preliminary data indicate that the preparation is not toxic in chronic experiments when used in the doses recommended for canning.

Conclusions

1. Preparations menips and β-propiolactone deserve particular attention in connection with their antimicrobic effect on the natural microflora of grape juice.

2. To inhibit the growth of yeast fungus in a monoculture on grape juice, it is necessary to use preparation menips in the amount of 0.003%, and for mold fungus -- 0.01-0.02%.

3. β-propiolactone in the amount of 1 ml per one liter of juice in combination with 5 minute pasteurization at a temperature of 50° inhibits the microflora and prevents canned fruit from spoiling.

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*Moldavian Institute of Epidemiology, Microbiology and Hygiene

4. Among the studied antibiotics, only nystatin had an inhibiting effect on the development of yeast in the course of 20 days.

5. According to the preliminary data of the MIEMG (Kishinev), preparation menips (methyl ether of 5-nitropyromucic acid) tested on rats in doses 10-100 times greater than those recommended for canning does not have any toxic effect on the organism of an animal.

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